Release of Cyanobacterial Metabolites Due to Preoxidation Processes

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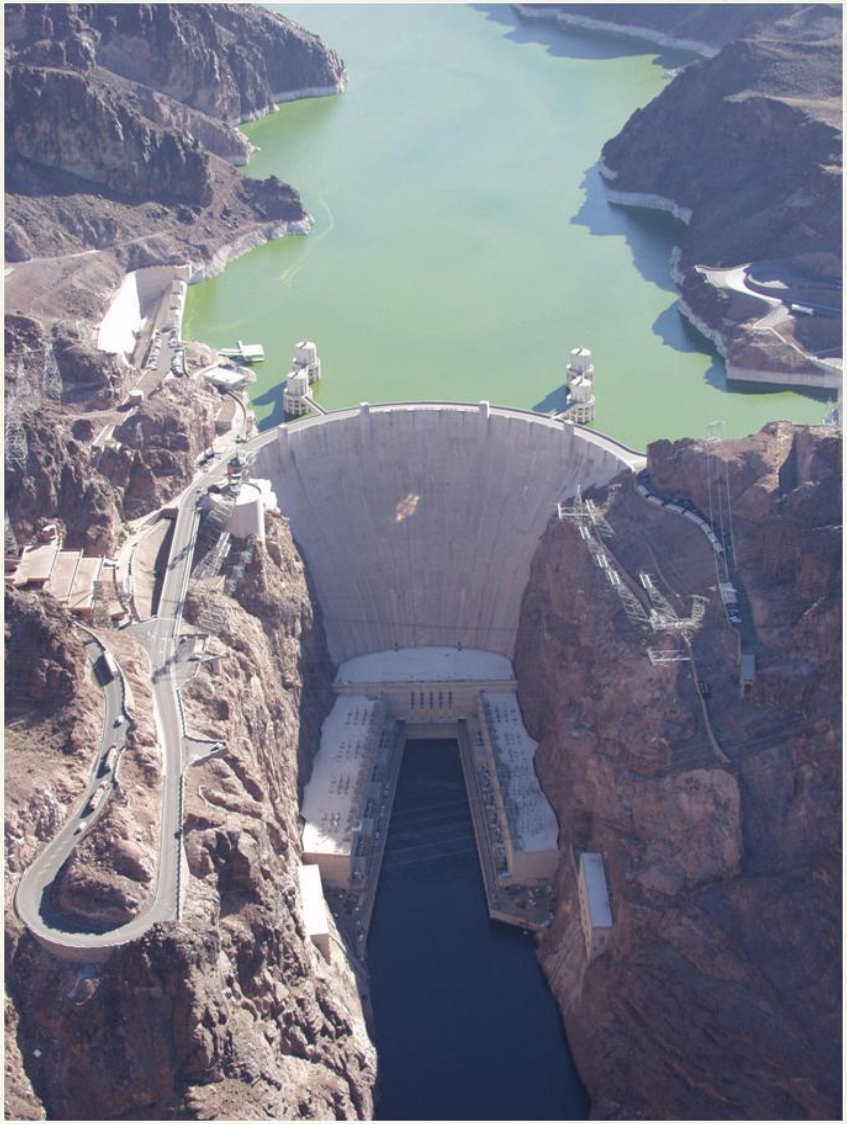
Lake Mead Ecosystem Monitoring Workgroup
November 13, 2014 – 1:00 PM

Presentation Outline

- Background on Cyanobacteria
- Cell Damage and Lysis
- MIB, Geosmin, Microcystin Release
- Summary
- Questions

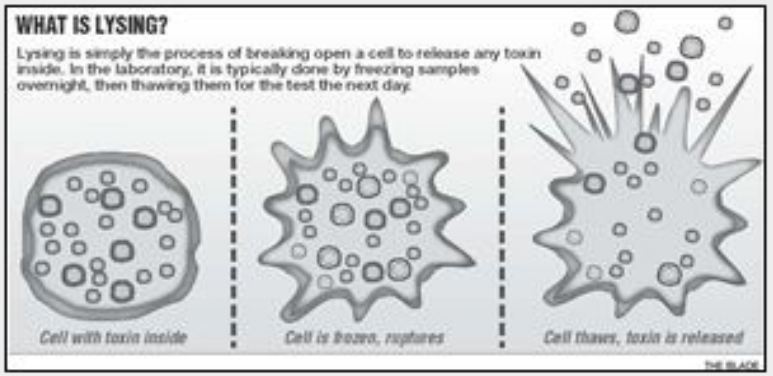


Frequency of Cyanobacteria Blooms

- Increased nutrient loading (i.e. wastewater, agricultural runoff)
- Climate change (i.e. temperature, hydrology)
- Quagga mussel infestation (e.g. selective filtering)

Think of lysing as the act of cracking open algal cells to get any toxin inside released.

In the case of microcystis, Lake Erie's most prevalent form of cyanobacteria, the goal is to get out any toxin that might be inside of it, called microcystin.



Enlarge

Cyanobacteria, also known as harmful blue-green algae, aren't actually algae.

But most people think it is because the bacteria look like and act like algae.

In the pretreatment process, a chemical called potassium permanganate is added at Toledo's water-intake crib so the raw lake water is easier to treat when it arrives at the city's Collins Park Water Treatment Plant about 6 to 12 hours later.

In the lab, samples of the finished product — the tap water the city is about to distribute — are typically frozen overnight, then thawed the next day to be tested.

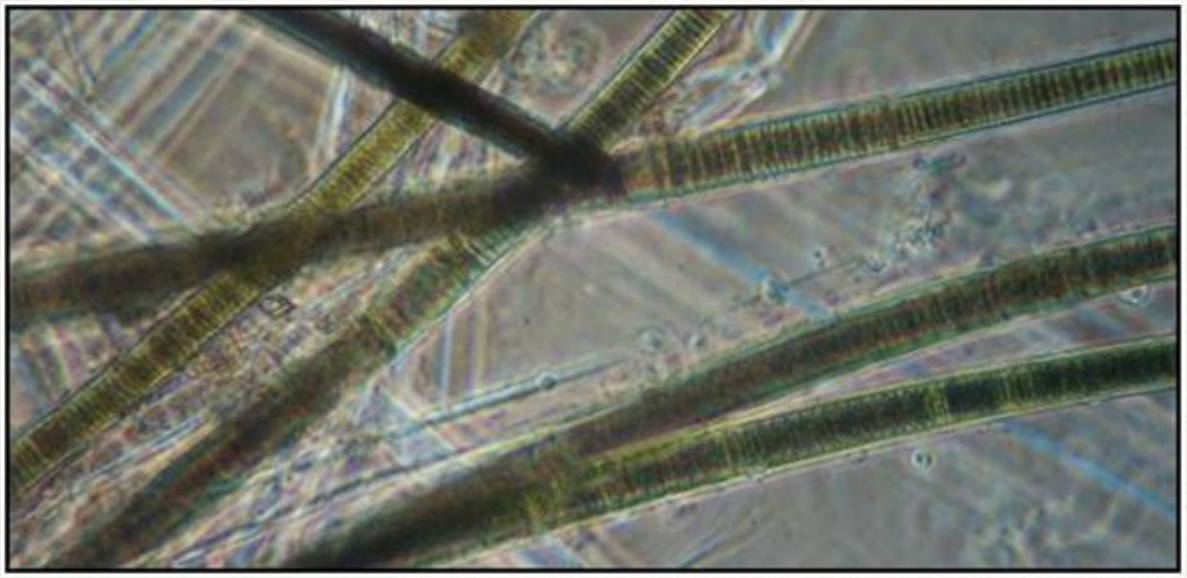
That lyses, or cracks open, any cells that might have eluded the treatment process and still have microcystin inside of them.

The lysing process was developed about 30 to 35 years ago, when cyanobacteria research was in its infancy, said Dave Deardorff, vice president of marketing and sales for Abraxis LLC, a company in Warminster, Pa., that manufactures the microcystin-detection kits many labs use.

Background on Cyanobacteria Treatment Research

- Treatment of **extracellular** metabolites has been studied for decades

- Minimal research on the release of **intracellular** (or cell bound) metabolites during oxidation process in drinking water treatment



Preoxidation is Common in Drinking Water Treatment

- Disinfection
- Control of Invasive Species (i.e. zebra/quagga mussel)
- Biofilm Control on Intake Pipelines
- Inorganic Contaminants
  - Iron/Manganese
- Organic Contaminants
  - Taste and Odor Compounds
  - Micropollutants

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- Tailored Collaboration Project
  - Southern Nevada Water Authority
  - University of Colorado at Boulder
  - Fluid Imaging Technologies

- 3 Primary Focus Areas
  - Cyanobacteria Cell Integrity
  - Release of Metabolites (i.e. cyanotoxins, taste/odor compounds)
  - Release of Disinfection Byproduct Precursors

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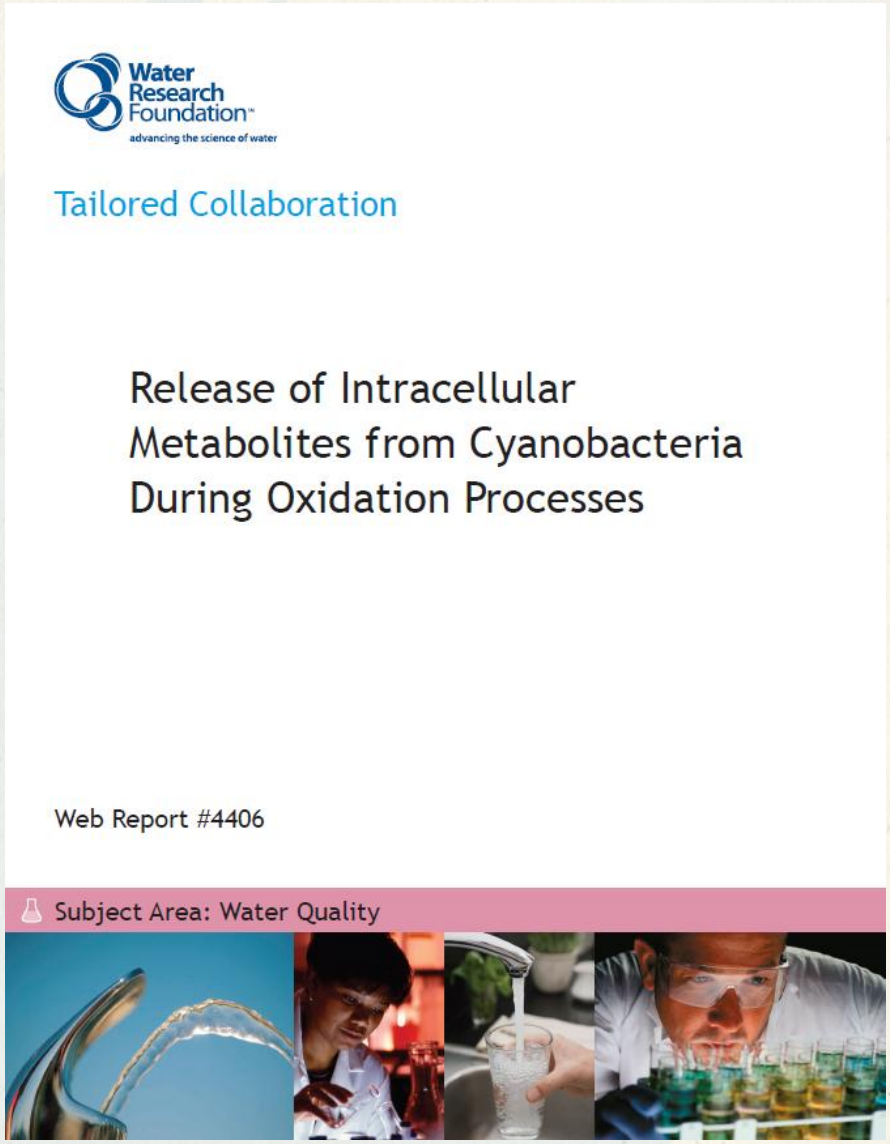

- Research conducted from 2011-2013
- Final Report Issued in May 2014 by the Water Research Foundation

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Chapter 1: Introduction

- Microcystin-LR Oxidation Kinetics (Source: Rodriguez, et. al., 2007)
  - Ozone: $k_{app}=4.1 \times 10^{5}\ M^{-1}s^{-1}$ — Fast
  - Hydroxyl Radicals: $k_{app}=1.1 \times 10^{10}\ M^{-1}s^{-1}$ — Fast
  - Chlorine: $k_{app}=33\ M^{-1}s^{-1}$ — Intermediate
  - Chlorine Dioxide: $k_{app}=1\ M^{-1}s^{-1}$ — Slow
  - Chloramine: $k_{app}< 1\ M^{-1}s^{-1}$ — Very Slow

- MIB/Geosmin Oxidation Kinetics (Source: Peter and von Gunten, 2007)
  - MIB: $k_{O3}=0.35\ M^{-1}s^{-1}$, $k_{HO}=5.1 \times 10^{9}\ M^{-1}s^{-1}$
  - Geosmin: $k_{O3}=0.10\ M^{-1}s^{-1}$, $k_{HO}=7.8 \times 10^{9}\ M^{-1}s^{-1}$

  - $FCl_2/ClO_2/NH_2Cl$ regarded as ineffective
    (Source: Glaze, et.al. (1990), Lalezary et.al. (1986))

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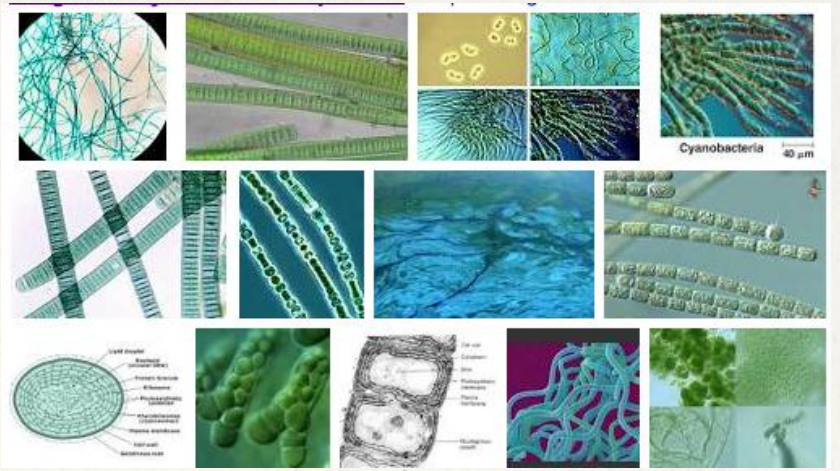
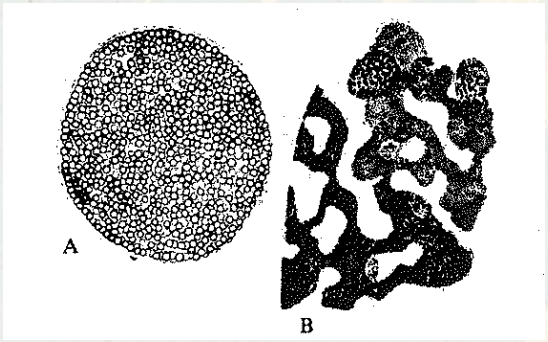
Chapter 2: Materials and Methods

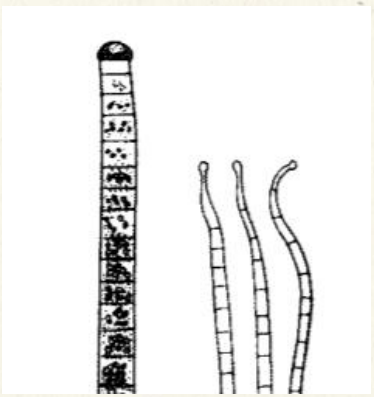
- Selection of Cyanobacteria

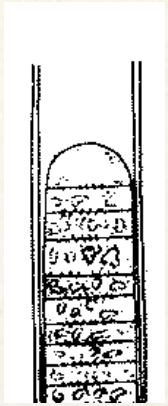


1. Availability of an axenic culture
2. Occurrence in source water supplies
3. Ability to produce metabolites of interest
4. Cell morphology

Microcystis aeruginosa (microcystin-LR)	*Oscillatoria sp.* (MIB, Geosmin)	*Lyngbya sp.* (Geosmin)

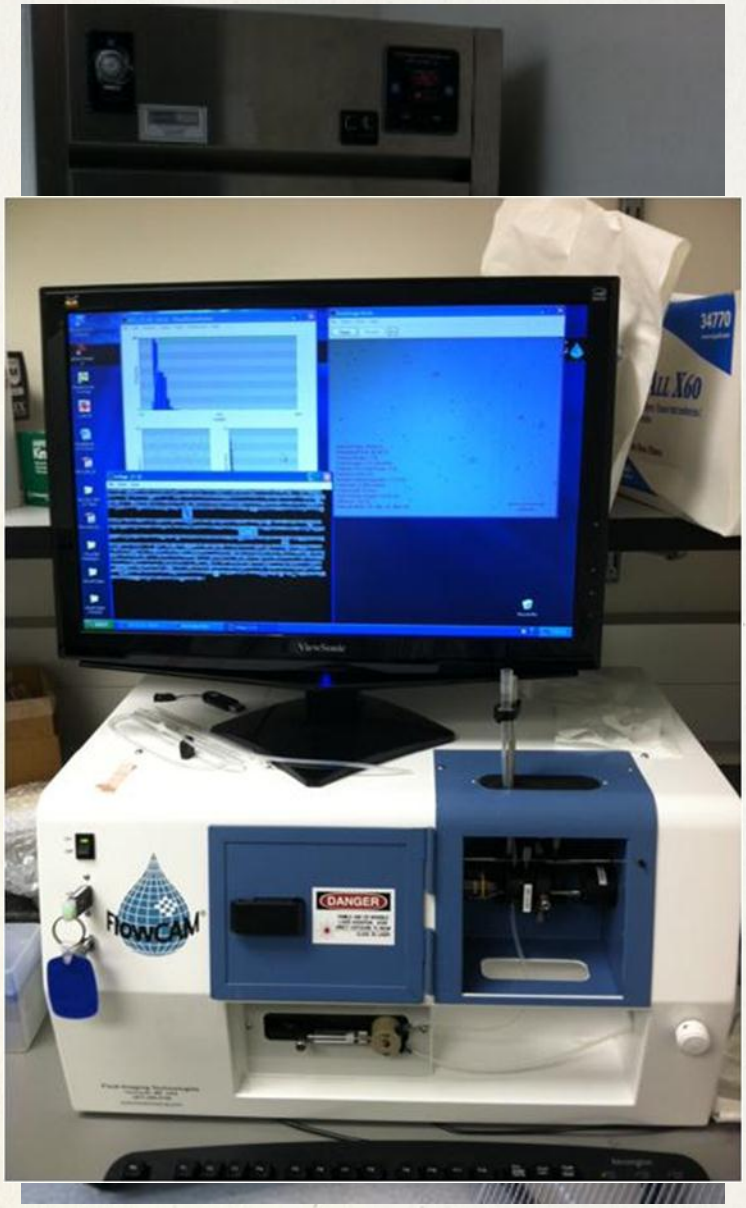




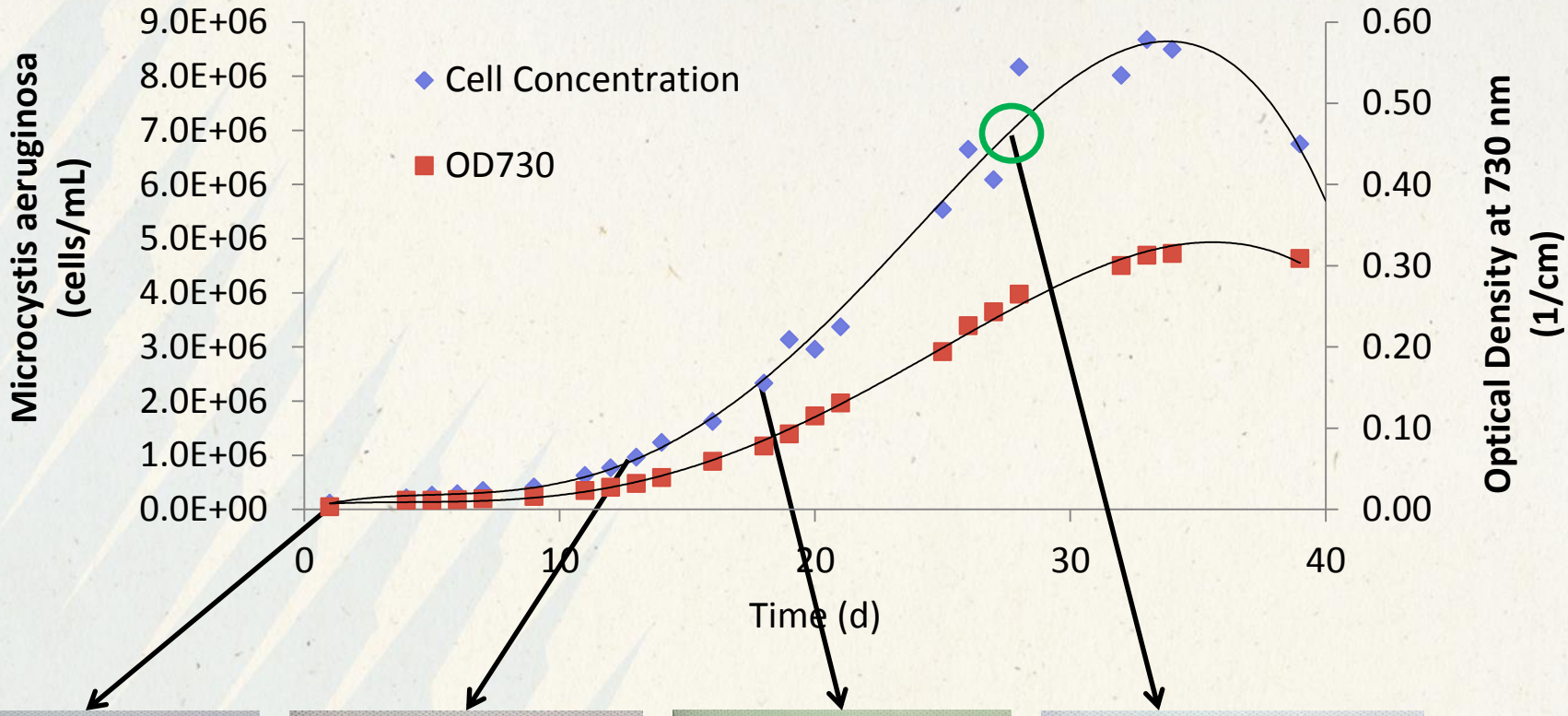


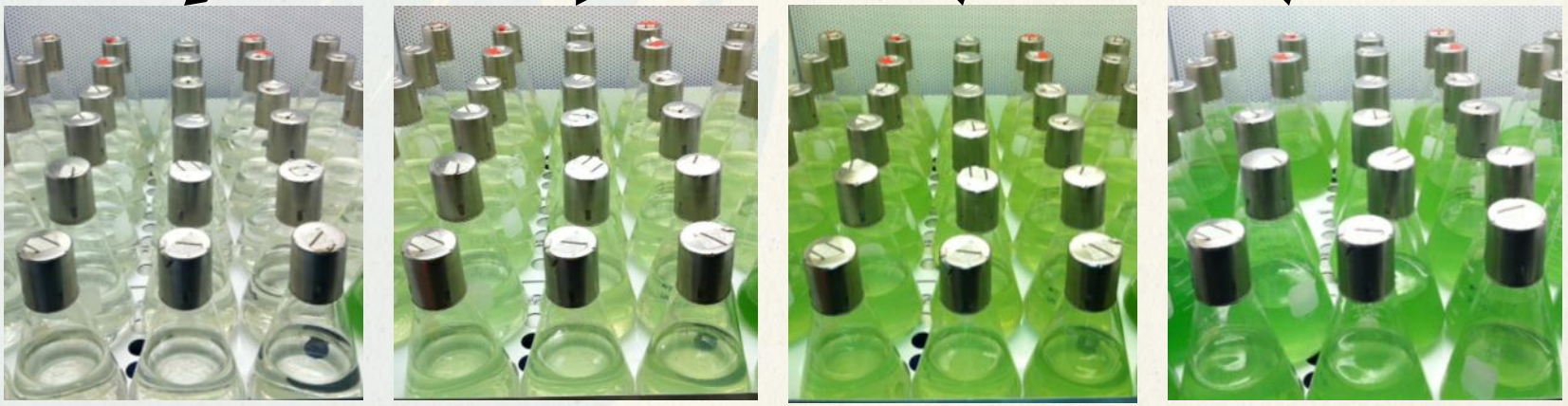
Culturing of Cyanobacteria

- Algal growth chamber
  - 12 hrs light/12 hrs dark
  - Constant temp = 22° C
  - Light Intensity = 2400 lux
- Culturing Methodology
  - 500 mL Erlenmyer Flasks
  - Growth Media (BG-11 or BOLD 3N)
- Growth Curve Development
  - FlowCAM
  - Optical Density at 730 nm



Development of Growth Curve





Preparation of Cell Suspension

Centrifuge

(1900 rpm, 14 min)

Cell Suspension

Discard Growth Media and EOM

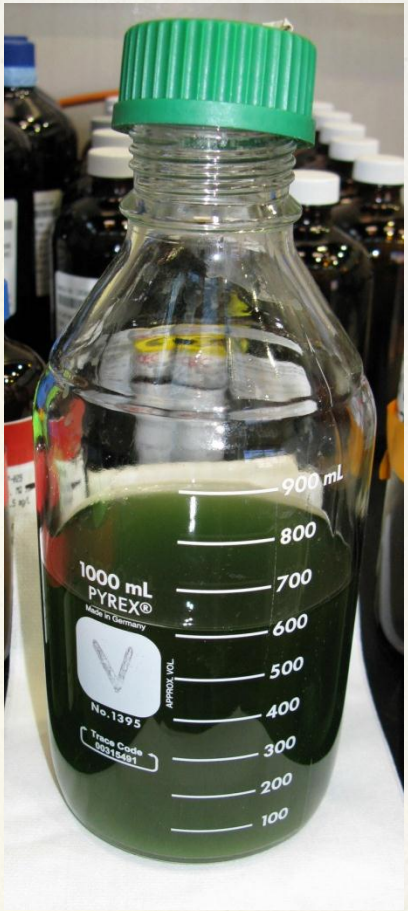
Dilute Cell Pellet with Buffered Milli-Q

Screen for FlowCAM Analysis

(<100 µm, OSC and LYN-only)

FlowCAM Quantification (Particles < 100 µm)

Cell Suspension Ready for Experiments



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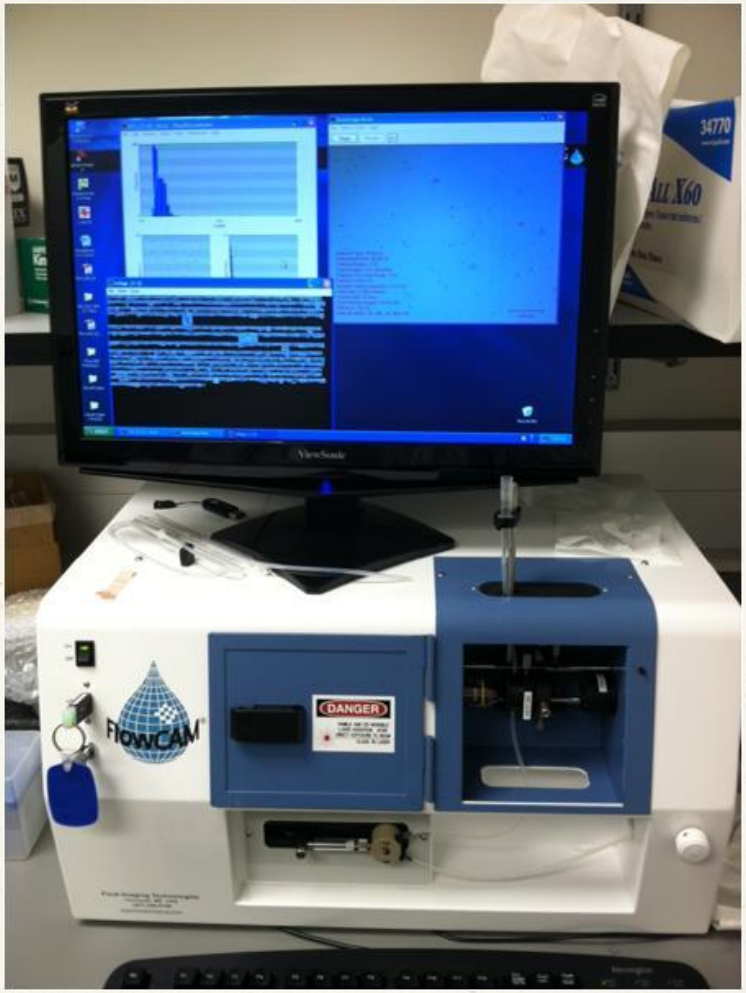
Chapter 3: Cell Integrity

- Added Cell Suspension to Colorado River Water (CRW)
  - *Microcystis aeruginosa* (MA) – 50,000 and 200,000 cells/mL
  - *Oscillatoria sp*. (OSC) – 1,600 and 3,200 cells/mL
  - *Lyngbya sp*. (LYN) – 800 and 1,600 cells/mL
- Oxidation Experiments
  - Ozone, Chlorine, Chlorine Dioxide, Chloramine
  - 5 Dosages of Each Oxidant
  - Residual Measurements

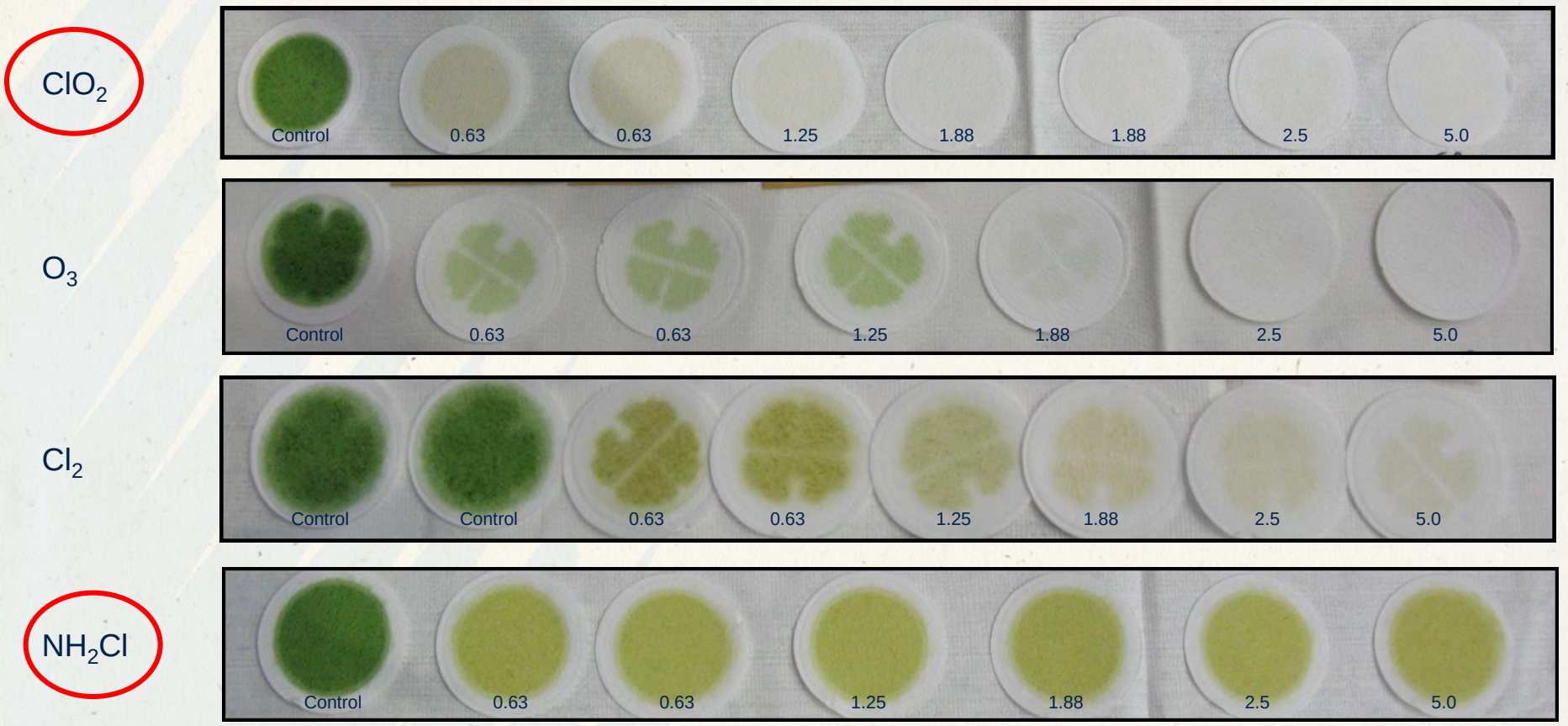
Cell Damage and Lysis - Approach

- Quantitative Assessment
  - Cell Damage
    - FlowCAM: Trigger Mode (TM)
    - Chlorophyll-a
  - Cell Lysis or Fragmention
    - FlowCAM: AutoImage Mode (AIM)
- Qualitative Assessment
  - FlowCAM: Digital and Binary Images



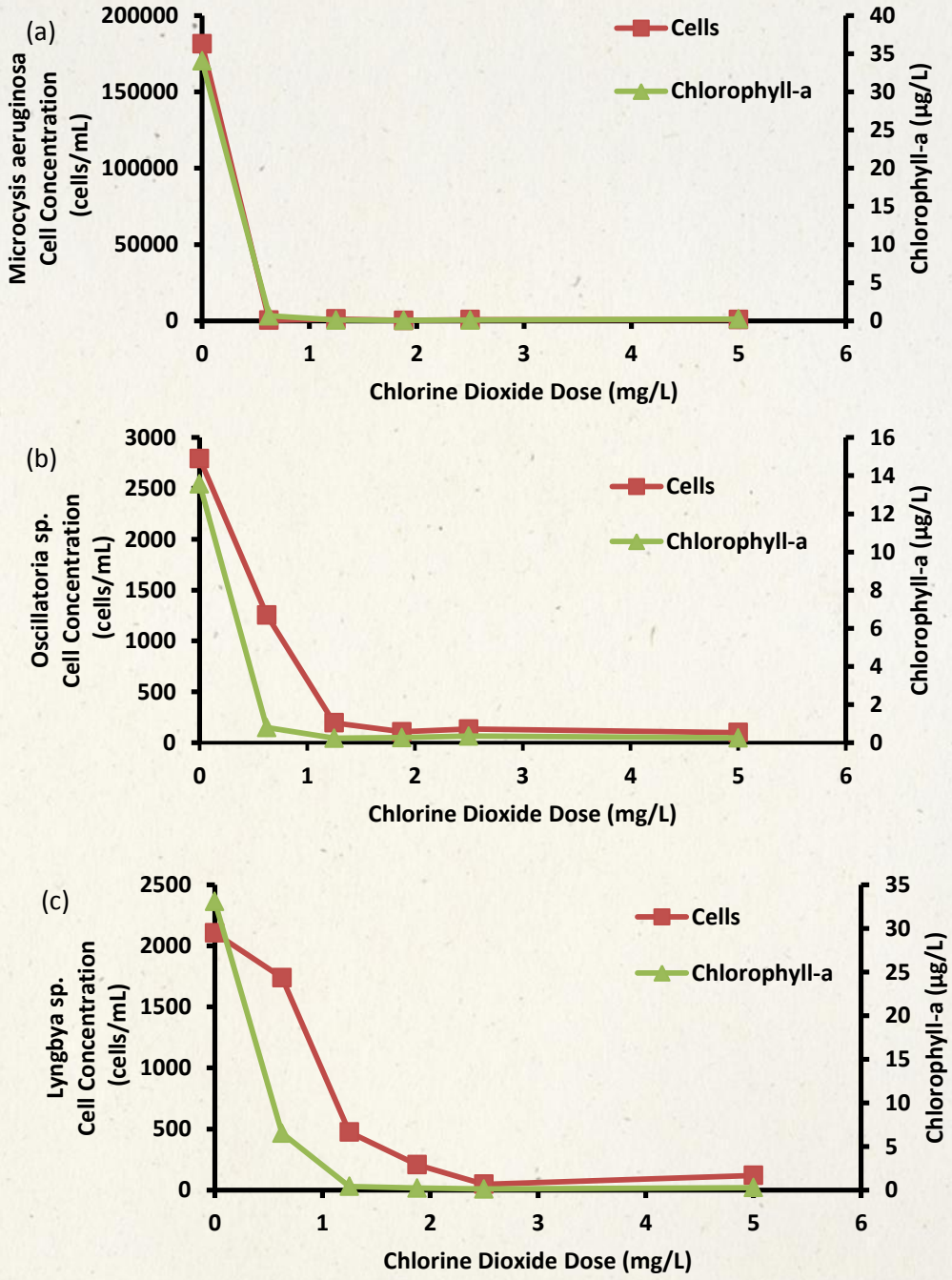
Degradation of Chlorophyll-a

***Microcystis aeruginosa* (200,000 cells/mL)**



Cell Damage by ClO_2

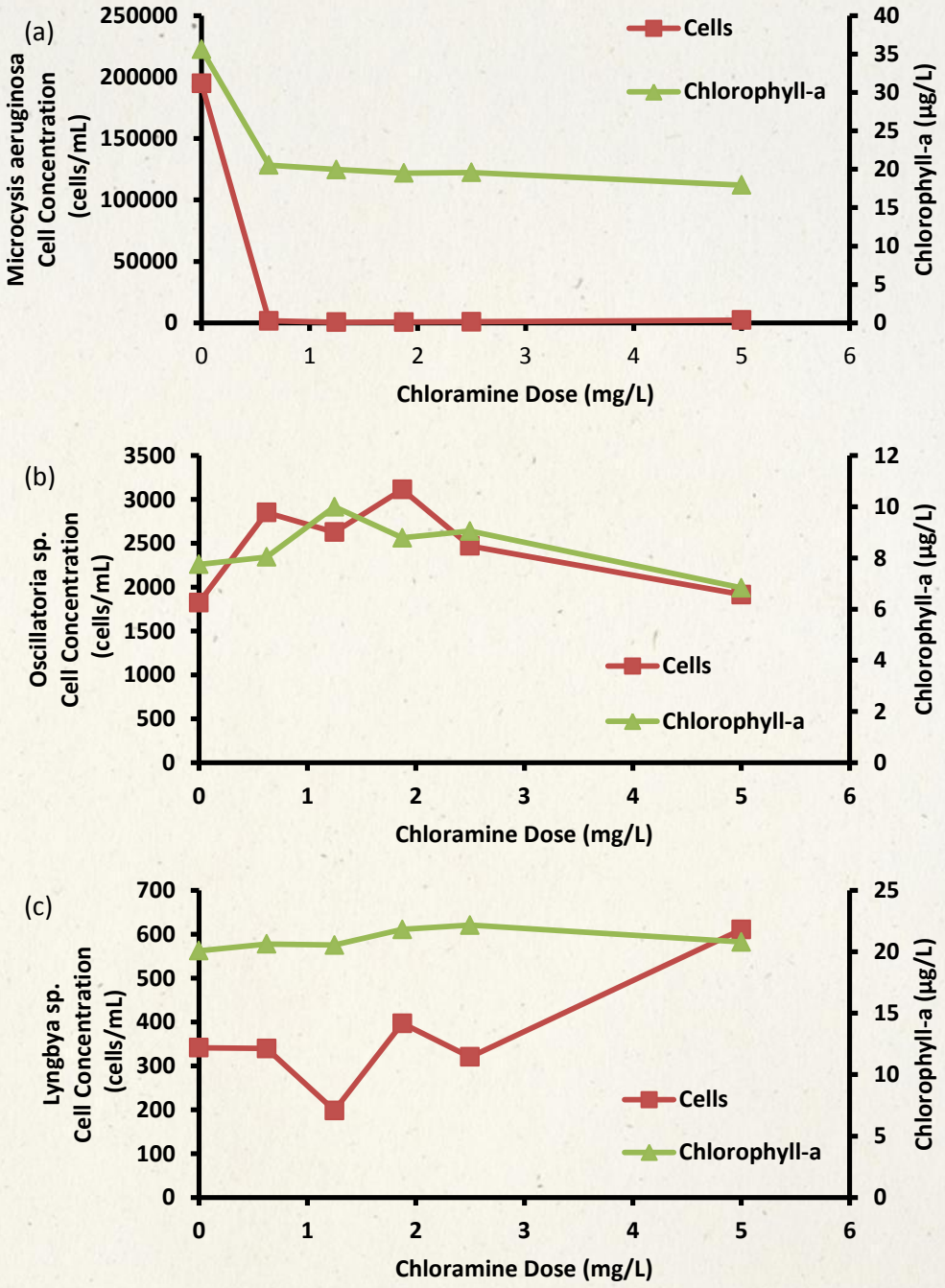
- Assessment
  - Trigger Mode Data
  - Chlorophyll-a
- MA rapidly damaged
  - (CT<386 mg-min/L)
- Dose response
  - OSC and LYN



Ref: Wert, E.C., et al., Water Research (2013)

Cell Damage by NH_2Cl

- MA rapidly lost cell integrity
  - CT<612 mg-min/L
  - Diffusion or reaction rate limited
  - Loss fluorescence intensity may have prevented triggering
- OSC and LYN
  - Photosynthetic pigments were unchanged
  - Minimal cell damage by NH_2Cl

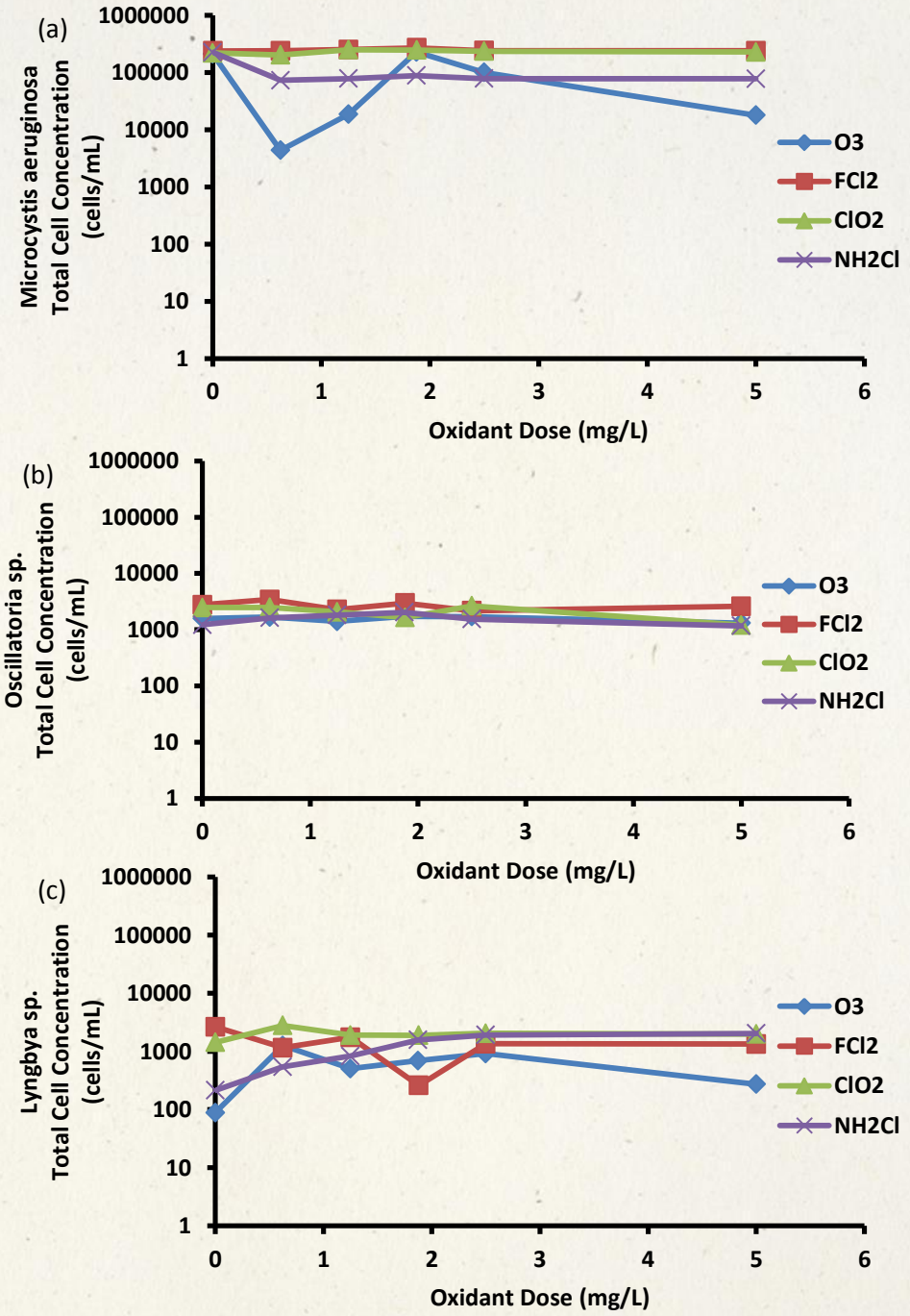


Ref: Wert, E.C., et al., Water Research (2013)

Cell Lysis

- Assessment
  - Auto Image Mode
  - All Particles

- Results indicate cells are damaged without resulting in complete lysis or fragmentation



Ref: Wert, E.C., et al., Water Research (2013)

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Metabolite Release - Approach

- Analysis of Filtrate from Oxidation Experiments

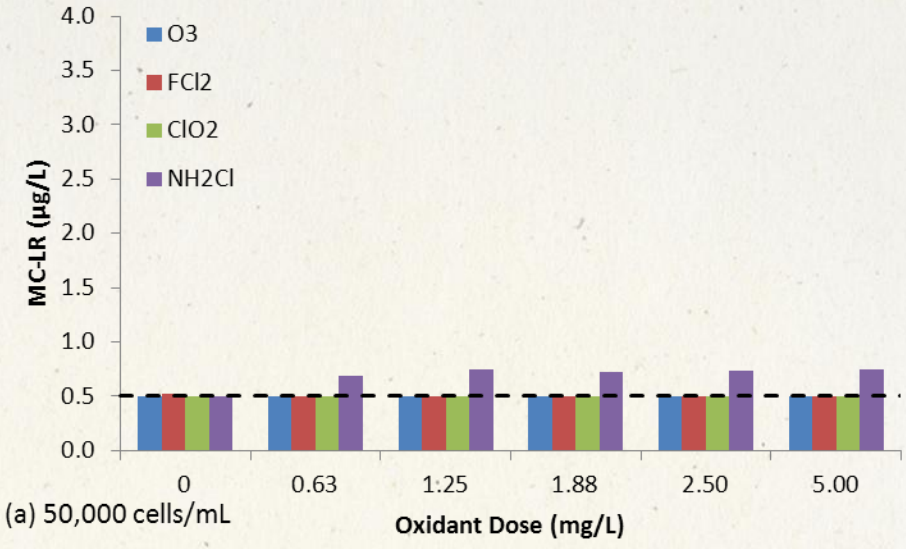
- Testing with *Microcystis aeruginosa*
  - Release of microcystin-LR
  - World Health Organization (WHO) provisional guideline of 1 µg/L
  - Included on USEPA Candidate Contaminant List 3 (CCL3)

- Testing with *Oscillatoria sp*.
  - Release of 2-methylisoborneol (MIB)
  - Threshold odor value of 6.3-15 ng/L

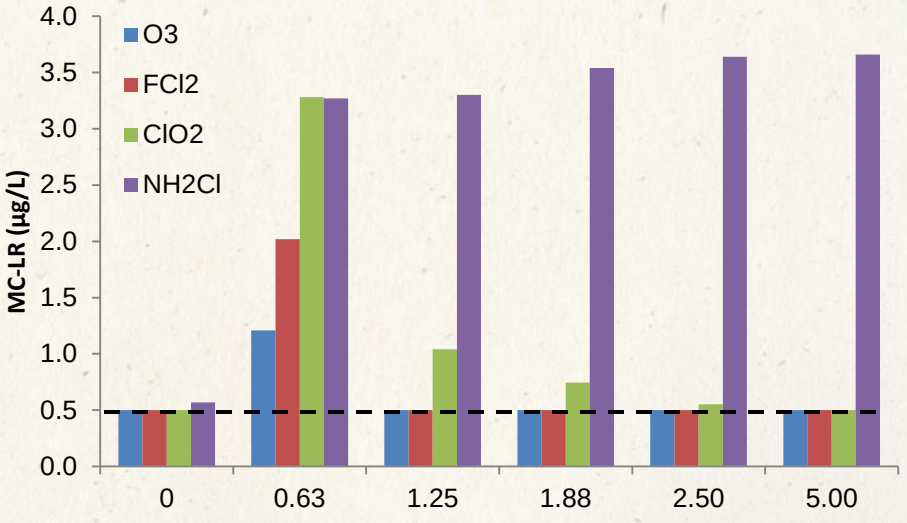
- Testing with *Lyngbya sp*.
  - Release of geosmin
  - Threshold odor value of 1.3-4.0 ng/L

MC-LR Release from MA

- Minimal MC-LR release occurred with 50,000 cells/mL
- MC-LR with 200,000 cells/mL
  - Release exceeded WHO guideline
  - Majority of release occurred at dosages < 0.63 mg/L
- Oxidation followed published kinetic information



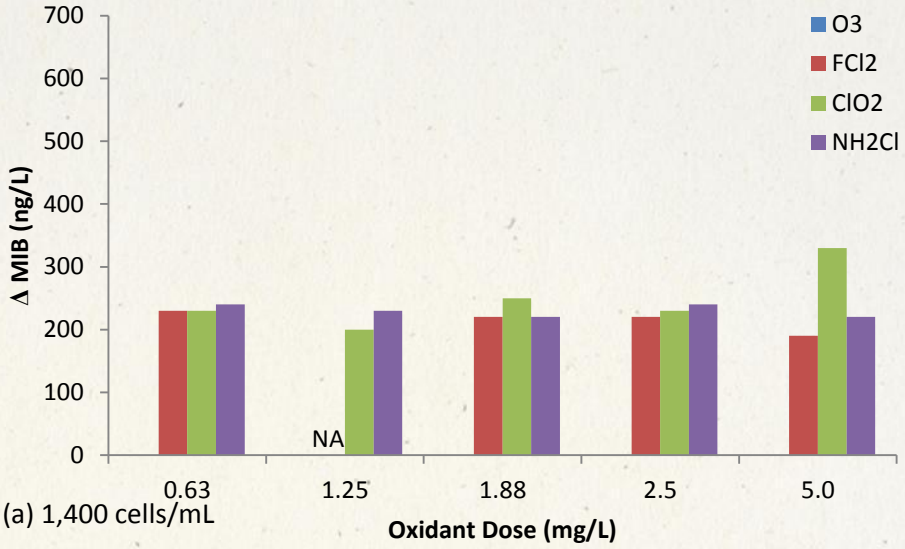
(a) 50,000 cells/mL

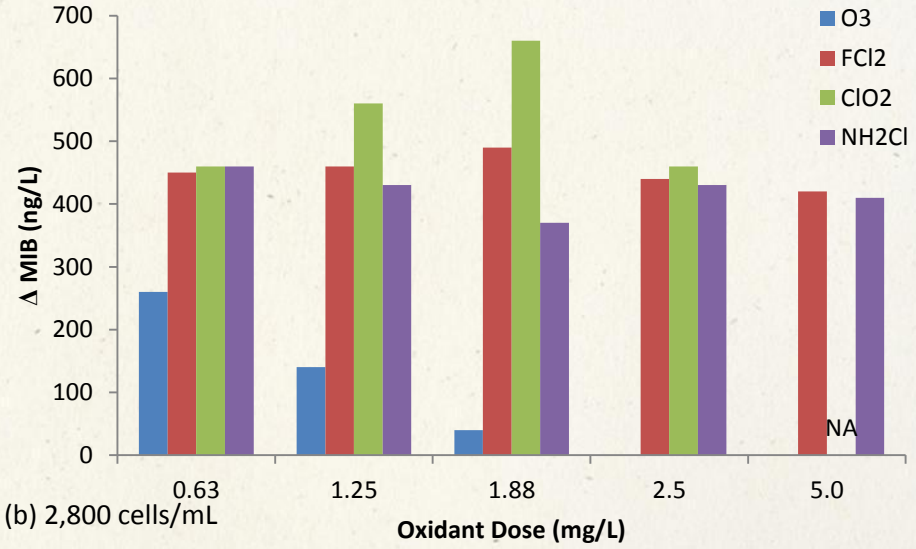


(b) 200,000 cells/mL

MIB Release from OSC

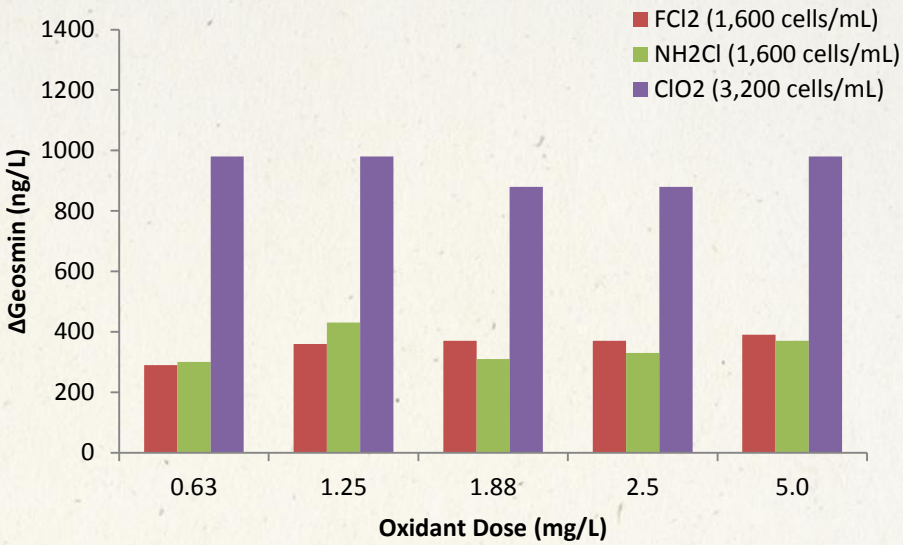
- MIB release exceeded threshold odor values (6.3-15 ng/L)
- Majority of release occurred at dosages < 0.63 mg/L
- Ozone/OH was able to oxidize released MIB
- Release and subsequent oxidation followed published kinetic information





Geosmin Release from LYN

- Geosmin release exceeded threshold odor values
- Ozone/OH was able to oxidize released geosmin
- Release and subsequent oxidation followed published kinetic information



Summary of Cell Integrity/Metabolite Release

- Ozone, Chlorine, and Chlorine Dioxide were able to damage all 3 species
- Chloramines damaged MA, but not OSC or LYN
- Oxidation did not result in complete cell lysis or fragmentation
- All oxidants resulted in the release of metabolites at low exposures
  - Physical release or due to oxidative stress
- Oxidation of extracellular metabolites dependent upon kinetics
  - Ozone and chlorine were able to oxidize released MC-LR
  - Hydroxyl radicals effective for MIB/geosmin

Acknowledgements

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  - Project Manager: Djanette Khiari
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